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Algal biofuel from urban wastewater in India: Scope and challenges

T.V. Ramachandra [a,b,c,*], Mahapatra Durga Madhab [a,b], Samantray Shilpi [a], N.V. Joshi [a]

[a] Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India
[b] Centre for Sustainable Technologies [CST], Indian Institute of Science, Bangalore 560012, India
[c] Centre for Infrastructure, Sustainable Transport and Urban Planning [CiSTUP], Indian Institute of Science, Bangalore 560012, India

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ABSTRACT

Rapidly depleting stocks of fossil fuels and increasing greenhouse gas (GHG) emissions have necessitated the exploration of cost effective sustainable energy sources focussing on biofuels through algae. Abundant wastewaters generated in urban localities every day provide the nourishment to nurture algae for biofuel generation. The present communication focuses on the lipid prospects of algae grown in wastewater systems. *Euglena* sp., *Spirogyra* sp. and *Phormidium* sp. were collected from selected locations of sewage fed urban lakes and sewage treatment plants of Bangalore and Mysore. The total lipid content of *Euglena* sp. was higher (24.6%) compared to *Spirogyra* sp. (18.4%) followed by *Phormidium* sp. (8.8%) and their annual lipid yield potential was 6.52, 1.94 and 2.856 t/ha/year, respectively. These species showed higher content of fatty acids (palmitate, stearate followed by oleic and linoleic acids) with the desirable biofuel properties.

Contents

* Corresponding author at: Indian Institute of Science, Energy Research Group CES, CES R 215, Bangalore 560012, Karnataka, India. Tel.: +91 80 23600985/22932506/22933099; fax: +91 80 23601428/23600085/23600683.
E-mail addresses: cestvr@ces.iisc.ernet.in, energy@ces.iisc.ernet.in (T.V. Ramachandra).

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1. Introduction

Greenhouse gas (GHG) levels in the atmosphere have increased during the post industrialization era by 25% and estimates reveal

that about three-quarters anthropogenic carbon-dioxide are due to burning of fossil fuels. Fast dwindling stocks of fossil fuels and consequences of GHG emissions have necessitated the exploration of cost effective sustainable energy sources [1–3]. Estimates reveal that fossil fuels such as oil, coal and gas would deplete in 35, 107 and 37 years, respectively [4], highlighting the impending energy crisis and the escalation of fuel prices with the dwindling of natural resources stock. Apart from this, nutrient accumulations in ecosystems due to indiscriminate disposal of liquid wastes have further enhanced GHG levels posing the threat of global warming and consequent changes in the climate.

Energy demand has increased with the spurt in economic activities, bringing along a change in the consumption pattern, which in turn varies with the source and availability of its energy, conversion loss and end use efficiency. The growing demand of burgeoning population coupled with developmental activities based on ad-hoc decisions have led to resource scarcity in many parts of India. In this context studies have shown that algae during their growth process synthesize carbohydrates during photosynthesis and then stock lipids variably. They act as a solar energy driven cell factory [5] converting CO_2–O_2 thereby reducing the atmospheric CO_2 while trapping nutrients from the environment. Cleaner, sustainable and cost-effective energy alternatives can be accomplished through the tiny oleaginous microorganisms like algae. Microalgae based biofuel are capable of meeting the global demand of fuels [5,6] with higher efficiency to overcome global warming, due to their higher growth rate requiring lesser cultivation area and higher biomass production [7]. Algae have good prospects as biofuel feedstock due to the higher photosynthetic efficiency, continuous year round productions and the ability to thrive in municipal wastewaters, marginal lands, etc. Fig. 1 compares the oil yield potential of various food crops with microalgae [1,8]. It is apparent that algae have the potential to produce up to ten times more oil per hectare land than other traditional biofuel crops like *Elaeis oleifera* (oil palm), *Jatropha curcas* (jatropha), (*Glycine max*) soybean etc. [1,8,9].

India meets about 75–80% of its total petroleum requirements through imports [10] causing a heavy burden on the foreign exchange. This has necessitated exploration for viable alternatives to diesel, for meeting the demand of transport, industrial and agricultural sectors [11]. The transportation sector in India consumes almost five times more diesel fuel than gasoline compared to other countries [9]. The annual consumption of diesel and gasoline from July 2011 to July 2012 is about 5.735 and 1.321 Million tonnes (Mt), respectively [12]. Diesel consumption has increased by almost two-folds in the last two decades [9]. This highlights the need for cost effective sustainable indigenous alternative to ensure food and fuel security in India. Moreover most of the urban municipalities are unable to handle wastewaters due to rapid urbanisation and lack of appropriate infrastructure. Wastewater generated in most urban areas is either untreated or partially treated and are let into nearby water-bodies. Wastewaters rich in C, N and P enhances the nutrient levels in the receiving water bodies (such as tanks, lakes and rivers) leading to eutrophication [13]. Conventional treatment plants (primary and secondary) are unable to remove nutrients particularly N and P. In this context, lagoons or algal ponds are attractive and viable solution for treating wastewater to the accepted levels [14,15]. Furthermore the sustained availability of nutrients through wastewaters allows prolific growth of micro algae, which can be harvested for extracting lipids. This would help in cost effective wastewater treatment at local levels, while giving an opportunity for clean energy production.

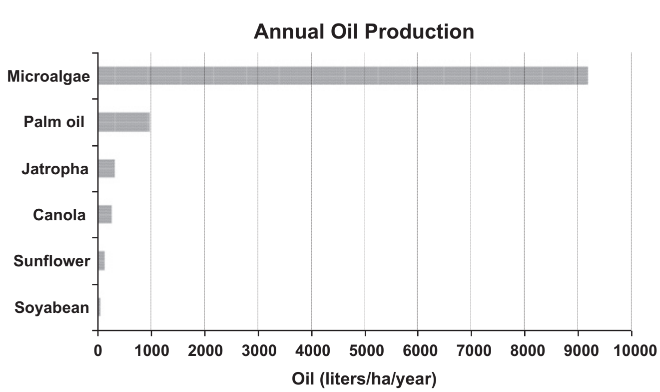


Fig. 1. Potential oil yield per hectare per year (adapted from Emily, 2009).

Many species of algae grow copiously in wastewater utilising abundantly available organic carbon and inorganic nutrients (N and P) and hence play an important remediation role in efficient removal of N and P [16,17]. The use of algae in wastewater treatment has been in practice since long [18] through the use of conventional oxidation (stabilization) ponds or the suspended algal pond systems (such as high-rate algal ponds HRAP) which have been highly effective [19,20]. In Algal systems, a significant amount of O_2 generation from photosynthetic algae helps in aeration avoiding mechanical aeration of the treatment pond and additional associated operational costs [17]. Oxygenation of ponds through algae also aids in bioremediation of organic and inorganic compounds by heterotrophic aerobic bacteria [21]. Furthermore, algal based remediation is environmentally amenable and sustainable as it does not generate additional pollutants such as sludge by-products and provides an opportunity for efficient recycling of nutrients.

Most of the research on algal wastewater treatment is based on the analysis of laboratory-based small scale and pilot pond-scale cultures and outdoor large open ponds [18,19]. The studies investigating the growth pattern of algae under a variety of wastewater conditions have focussed on evaluating the potential of algae for removing N and P, and in some instances metals from wastewater [16]. However the information pertaining to the potential of wastewater algae as an alternative energy options is scant. Experimental studies addressing the factors to maximise algal biomass production and harvesting will benefit the evaluation of wastewater-grown algae as a potential biofuel option.

The inadequacy of fossil fuels and increasing oil prices along with the increasing levels of greenhouse gas emissions poses inevitable threats to global environment, and necessitates our dependencies on alternative energy sources as algal fuel [1]. The concept of algal usage as biofuel feedstock, followed by lipid extraction and transesterification have recently been realised as the step towards exploring sustainable options for meeting the energy demand [9]. Steady-state nutrient supply and ways of extracting lipid are the most challenging stages in realising algae based biofuel [22]. The algal biofuel production involves a series of unit processes including species selection, cultivation, biomass harvesting, and lipid extraction. This requires a sound understanding of algal downstream processing with the process optimisation for successful commercial exploitation.

Cell disruption is the most crucial step for recovering intracellular products from algae that enhances the lipid extraction efficiency [23]. The efficiency of the disruption methods depends upon the ways to lyse the targeted cell by

effectively breaking the cell wall components. Cell disruption can be done through mechanical, physical, chemical, enzymatic or combined methods. Various cell disruption techniques include bead beating, sonication, microwave, high-pressure homogenisers, autoclaving and addition of hydrochloric acid, sodium hydroxide, or alkaline lysis [24,25]. The selection of solvents for extracting metabolites as fatty acids is essential as it causes alterations to the cell membrane to enhance the movement of globules out of the algal cells [26]. Thus the bio-chemical properties of cell walls play a vital role in the solvent extraction process. Generally a thick cell wall is commonly found in microalgae rendering them suitable to survive in harsh conditions. The characteristics of algae plays an important role for lipid recovery, that would be from cells, which are strong enough to withstand shear stresses at the same time, can lyse easily to obtain a reasonable lipid yield.

Earlier studies indicate that bead beating is the most effective cell disruption method for *Botryococcus braunii* [27]. Nevertheless bead beating induces direct mechanical damage to the cell as they are subjected to high stress produced by high pressure, abrasion during rapid agitation by glass beads [28]. Bead beating has been suggested for cell disruption with good potential for industrial scale up [23]. However sonication is one of the most widely used laboratory disruption technique, where high acoustic power inputs (sound waves of frequency higher than 15–20 kHz) are used to disrupt the cell wall [28]. Sonication is said to be the most applicable and efficient method of lipid extraction from microalgae [25].

The objective of the present work is to explore the lipid prospects from microalgae of urban wastewater ponds, which involves:

(a). The analysis of conditions required for higher algal growth in municipal wastewater;
(b). determination of algal lipid potential in urban wastewaters;
(c). comparative analysis of different cell disruption methods to find the best method for lipid extraction for improved yield of essential fatty acids present in three different wastewater grown algal species; and
(d). analysis of composition of fatty acids in selected algal species and their suitability as biofuel.

2. Materials and methods

2.1. Algal screening and selection

Algal samples were collected as a part of biomonitoring programme from sewage fed lake and sewage treatment plants. *Spirogyra* sp. and *Phormidium* sp. (macroalgae) were predominant in Varthur lake, Bangalore. *Euglena* sp. (microalgae) dominated in the sewage treatment plants at Mysore STP. Algal species were identified using standard keys [29] based on their external appearance, colour, morphological characteristics, size, habitat, orientation of chloroplast, cellular structure and pigments etc. Wastewater samples collected were concentrated by centrifuging 15 ml volume. Algae were enumerated using 3 replicates of 20 μl of the concentrated sample where it was placed over the slides with cover slips for microscopy observations. The numbers of algae present were calculated as per Eq. (1):

$$\text{Numberofalgalcells/ml} = \frac{\text{Numberoforganismscounted}}{\text{Numberofreplicates}} \quad (1)$$

2.2. Characterisation of the growth environment and water quality

Water samples (1 l) in triplicates were collected from the chosen sampling locations between 9 and 12 a.m. Sampling was done always from the sun facing side of the banks/shore avoiding shaded areas. pH, water temperature, electrical conductivity (APHA method 205), total dissolved solids (TDS), salinity, dissolved oxygen (DO), dissolved free carbon-dioxide (free CO_2) and turbidity were measured on-site using the standard methods. 1-l sub-sample was analysed according to standard methods [30], total biochemical oxygen demand over 5 days (BOD_5) (APHA method 507), filtered BOD_5 (APHA method 507, GF/C filtered (1.2 μm pore) with subsequent addition of 1 ml of unfiltered sample), chemical oxygen demand (COD) (APHA, 5220C), suspended solids (SS) (APHA method 209c), SAR (APHA 1206), turbidity (Hach turbidity meter, APHA method 214a), ammoniacal nitrogen (APHA method 417a), total Kjeldahl nitrogen (TKN) (APHA method 420a, total nitrogen (TN by calculation of TKN+TON), Phosphates (APHA, 4500-P D). All sample bottles were acid-washed. The samples were stored in a cool place until transfer (within few hours) to the laboratories. In addition, visual clarity/transparency of the lake/STP wastewater was measured as with secchi disk.

2.3. Algal density and harvesting of algal biomass

Samples collected from field were weighed for total dry wt. Quantification for unit area/volume was done taking 1 m × 1 m quadrate for microalgae and 10 l volume for microalgae. The macroscopic algal biomass was collected from a quadrate of 1 m × 1 m from the lakes. These floating algae were carefully washed at site and were transferred to the laboratory for further separation. After microscopic analysis, the samples were washed thoroughly with deionised water and were concentrated by centrifuging at 3292*g* for 20 min for further lipid extraction. The pellet was scrapped carefully using spatula and was exposed to drying at room temperature. The samples were preserved for further use.

2.4. Algal oil extraction

2.4.1. Cell disruption

Algal cells after concentration were disrupted using techniques such as (1) Bead beating (bead diameter: 0.75–1 mm) using magnetic stirrer (Shalom Instruments), (2) Sonication using a ultrasonic bath (frequency 35 kHz) for 30 min, and (3) Mortar–pestle Maceration (macerated for 10 min, manually).

2.4.2. Lipid extraction

Lipids were extracted from solvent mixture as per modified Bligh and Dyer's method [31]. 0.5 gm of dry algal biomass was mixed with a solvent mixture of chloroform and methanol in the ratio of 2:1 (v/v). The organic chloroform layer was separated and was evaporated using a rotary evaporator with a water bath temperature of 60 °C. The lipid classes were separated by one-dimensional thin-layer chromatography (TLC) using TLC plates (10 × 10 cm, 0.25 mm thickness, Merck, Darmstadt, Germany) coated with silica gel. The solvent system used for elution of lipids was a combination of petroleum ether: diethyl ether: acetic acid in a ratio of 70:30:1 (v/v) [32]. The bands were visualized after staining the TLC plates with iodine vapours as per standard TLC protocols [33]. The triacylglyceride (TAG) layer was immediately and carefully scrapped out and was processed for total lipid extraction.

2.4.3. Methylation

The fatty acids were analysed by methylation of lipid samples using Boron trifluoride–Methanol (BF_3–MeOH) as per established FAME protocols, where BF_3–MeOH converts fatty acids to their methyl esters in 2 min. BF_3–MeOH is the most commonly used catalyst used for FAME preparation and has also been adopted by American Oil Chemist's Society (AOCS) [34] (Method Ce 2-66). The extracted samples were heated at 60 °C for 15 min. It was then cooled in ice-bath for 5 min followed by the addition of 1 ml water and hexane, respectively. After settling, the top hexane layer was removed and washed using anhydrous sodium sulphate for further purification. The sample was then injected into the gas chromatography column using hexane as a carrier gas. The methyl esters formed were assessed via gas chromatography (GC) and identified using mass spectroscopy (MS) on the basis of their retention time and abundance.

2.4.4. Fatty acid composition using GC–MS

The component of fatty acids was assessed through gas chromatograph (Agilent Technologies 7890C, GC System) using detection by Mass spectrometry (Agilent Technologies 5975C insert MSD with triple-axis detector). The injection and detector temperature were maintained at 250 °C and 280 °C, respectively (ASTM D 2800). 1 μl volume of sample was injected into the column, whose initial temperature was maintained at 40 °C. After 1 min the oven temperature was raised to 150 °C at a ramp rate of 10 °C/min. The oven temperature was then raised to 230 °C at a ramp rate of 3 °C/min and finally it was raised to 300 °C at a ramp rate of 10 °C/min and this temperature was maintained for 2 min. The methylated sample was loaded onto silica column with helium gas as carrier in splitless mode. The total run time was calculated to be 47.667 min. Fatty acids were identified by comparing the retention time obtained to that of known standards.

Table 1
Growth environment of the selected algal species.

Parameters	Units	Sampling locations (1–3)		
		Euglena (1)	*Spirogyra* (2)	*Phormidium* (3)
pH		7.78 (±0.43)	8.4 (±0.35)	8.3 (±0.23)
Temperature	°C	28.1 (±1.60)	24.5 (±0.08)	23 (±0.75)
Salinity	mg/l	566 (±18.19)	423 (±14.2)	428 (±12)
Total diss. solids (TDS)	mg/l	968 (±62.36)	792.85 (±17)	736.75 (±8)
Total suspended solids (TSS)	mg/l	389 (±153.17)	103 (±24.5)	277 (±34.6)
Turbidity	NTU	176 (±24.5)	81.1 (±8.4)	104.8 (±12.7)
Electical conductivity (EC)	μS/cm	1138 (±32.96)	888 (±14)	862 (±22)
Dissolved oxygen (DO)	mg/l	5.8 (±3.72)	18.2 (±2.8)	0 (±00)
Chemical oxy. demand (COD)	mg/l	362.7 (±90.88)	104.44 (±7.7)	122.22 (±3.85)
Filterable COD	mg/l	109.3 (±14.84)	74.7 (±12.2)	88.4 (±14.4)
Biochemical oxy. demand (BOD)	mg/l	224 (±78.50)	62.64 (6.08)	86.58 (±3.69)
Filterable BOD	mg/l	36.2 (±4.59)	18.6 (±4.16)	31.6 (±8.09)
Nitrates	mg/l	0.181 (±0.01)	0.77 (±0.01)	0.33 (±0.02)
Nitrites	mg/l	0.0138 (±0.03)	UD	UD
Ammoniacal-N	mg/l	20.54 (±.23)	9.54 (±3.42)	16.66 (±1.27)
Total kjeldahl nitrogen (TKN)	mg/l	43.68 (±10.96)	22.4 (±3.75)	18.6 (±4.68)
Inorganic phosphates	mg/l	6.75 (±0.41)	3.06 (±0.24)	2.39 (±0.41)
Total phosphates (TP)	mg/l	7.81 (±1.72)	13.1 (±0.75)	9.92 (±1.46)
Alkalinity	mg/l	560 (±30.55)	346.67 (±5.77)	326.67 (±5.6)
Carbonates	mg/l	40 (±23.09)	20 (±10.5)	20 (±0)
Bicarbonates	mg/l	520 (±41.6)	326 (±24.5)	306 (±17.2)
Ca	mg/l	56 (±3.51)	66.35(±1.15)	68.08 (±3.46)
Mg	mg/l	36 (±2.52)	24.14 (±1.07)	28.16 (±2.43)
Total hardness	mg/l	288 (±2.31)	193.33 (±1.5)	225.33 (±1.15)
Na	mg/l	208 (±3.51)	152.8 (±2.4)	151 (±4.6)
K	mg/l	44 (±2)	38.3 (±0.5)	34 (±0.5)
Chlorides	mg/l	122 (±1.73)	104.13 (±0.82)	112.18 (±1.42)
Sodium absorpt'n ratio (SAR)		30.66 (±2.5)	22.71 (±14.2)	21.76 (±7.6)
ORP	mV	58 (±10.6)	28 (±8)	−178 (±24)
Total organic carbon (TOC)	mg/l	65	6	14

Table 2
Algal lipid dry weight content in different wastewaters.

Municipal wastewater	Algal species	Lipid (% dry wt)	Reference
Primary treated	Mixed sp.	28	[60]
Secondary treated	*Scenedesmus obliquos*	31.4	[62]
Secondary treated	*Botryococcus brauni*	17.85	[65]
Primary treated + CO_2	*Micracitinium* sp., *Actinastum* sp. *Chlorella* sp. (mixed)	9	[66]
Centrate	*Chalmydomonas reinhardtii*	25.25	[61]
Facultative pond (STP)	*Euglena* sp.	24.6	Present study (2011)
Varthur Lake	*Spirogyra* sp.	18.4	Present study (2011)
Varthur Lake	*Phormidium* sp.	8.8	Present study (2011)

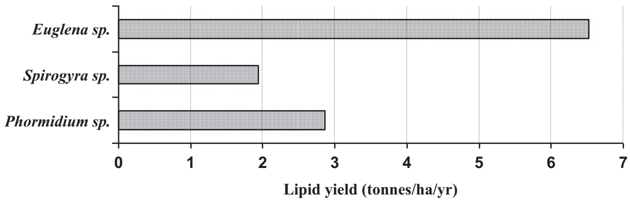


Fig. 2. Annual lipid yield of the select algal species in urban wastewaters.

3. Result and discussion

3.1. Wastewater characteristics

Water samples collected from wastewater pond were rich in nutrients. *Euglena* sp. were abundant in wastewater with Amm-N level of 20 mg/l with higher BOD and COD values of 224 and 362, respectively, out of which 50% i.e., 115 mg/l was only algal BOD (Table 1). These species contributed to higher TSS load of 389–465 mg/l. The TKN values were 44 mg/l with a very minimal nitrification occurring with, nitrate and nitrite values of 0.18 and 0.01 mg/l, respectively. It was observed that *Euglena* sp. were responsible for BOD removal of ~60%, TSS removal of 80% and Amm-N removal of 35%. The wastewater of the treatment ponds showed an algal count of 4.78×10^5 cells/ml. Higher algal cell densities were found on the top 40 cm layer of the treatment plant. Earlier studies have been performed on *Scenedesmus* sp., *Botrycoccus* sp., *Chlorella* sp., *Chlamydomonas* sp., *Micracitinium* sp. etc. from primary or secondary treated municipal wastewaters for their lipid content (Table 2).

Blooms of *Spirogyra* sp. were observed in the shallow waters at a comparatively lower level of Amm-N (9.54 mg/l) in the wastewater fed Varthur lake (Table 1). BOD and COD of the region were moderately high (Table 1). Dissolved oxygen levels of ~18 mg/l (Mid-day) were higher due to algal photosynthesis with reasonably high phosphate levels (Table 1). *Phormidium* sp. were prevalent as floating masses and to a smaller extent attached to the substratum near the outlets of Varthur lake in a highly reducing environment having an ORP of −214 mV with a slightly higher BOD, COD and lower Amm-N values (Table 1).

3.2. Lipid potential and algal-biomass productivities in urban wastewater systems

In case of *Euglena* sp., 10 l of the wastewater sample concentrate yielded 5.1 g of algal biomass on a dry weight (wt) basis. Considering the period for the peak exponential growth as seven days, and the oil content as 24.6%, the area required for wastewater treatment as 10 ha, the lipid potential of *Euglena* sp. under such high ammonia conditions is 6.52 t/ha/annum (Fig. 2). Similarly, from a quadrate of 1 m^2 from Varthur lake 480 g wet wt of *Spirogyra* sp. was collected that yielded 20.37 g of dry wt. The total lipid content observed was 18.4%. For a sampled area of 1 m^2 plot 3.74 g of oil could be derived and the estimated lipid potential is 1.94 t/ha/annum (Fig. 2). *Phormidium* sp. are mostly found to be near the shallow regions and are mostly as floating masses and also with forms attached to substratum. From a plot of 1 × 1 m quadrate, 257.5 g wet wt was collected, dry wt content was 62 g and the total lipid content was 8.86%. Oil yield of these species is about 54.93 kg/ha and the lipid potential is about 2.856 t/ha/annum.

The mean insolation for India is about 5 kW h/m^2/day with the range 4–7 kW h/m^2/day [35]. In terms of energy it is expressed as 6.57×10^9 J/m^2/year. Therefore the amount of solar energy falling over each hectare of land equals to 6.57×10^{13} J/ha/year. Assuming that diesel uniformly contains 42 GJ/tonnes of energy and considering the current field productivities of the wastewater samples and lipid content as 20% on a dry wt basis the energy equivalent for the diesel component of the biomass in dry wt figures to 2.1×10^{11} J. Thus the photosynthetic efficiency on lipid is only 0.31%. The algal biomass yield from the wastewater under such conditions, accounts to 26 t/ha/annum and the annual productivity of lipids is around 5 t/ha. The total wastewater generated in the country is approximately 1,70,000 MLD based on the national wastewater statistics along with the census data for population [36]. According to the current growth rates of algae in lagoons and treatment ponds, an area of ~8,45,000 ha is required to convert the wastewater nutrients into algal biomass and consequently lipid for catering to the growing energy requirement. The lipid potential for the country based on the algae potential state-wise is depicted in Fig. 3. Availability of wasteland throughout the country as per the data obtained from National Remote Sensing Centre, Hyderabad [37] gives scope for creating algal ponds to treat wastewater and also to generate oil. The ratio of the wasteland availability to the land requirement for algal pond construction for lipid production has been illustrated in Fig. 3. The higher the value, the higher is the feasibility of the acquirement of the land for simultaneous wastewater treatment and lipid production through wastewater fed algal ponds.

It was estimated that under present environmental conditions, about 5 t/ha/year can be achieved annually from wastewater algal ponds considering ~20% lipid content on a dry wt basis. Other studies conducted at the NREL projected 50–300 t/ha in closed PBR's [38]. However studies conducted elsewhere predicted 22.87–137.25 t/ha/year at about 30–70% oil on a dry wt basis [5]. Both the projections mentioned above are far higher that can be really achievable in field conditions.

3.3. Total lipid content

Earlier studies have revealed varied lipid accumulation and lipid content under various environmental conditions. The lipid content ranges from 20% to 50% in *Scenedesmus* sp. at varied N and P concentrations [39] and 42% in *Chlorella* sp. grown in wastewater in a semi-continuous mode with effective COD, NH_3–N and P removal [40]. The total lipid content of *B. braunii* was reported to range from 26% to 86% of the dry weight [41].

The total lipid contents for the algae analysed in this study ranged from 6.18% to 31% of the dry weight. Lipid content for *Phormidium* sp. (6.2–11.5%) was comparatively lower than that of *Spirogyra* sp. (18.4–20%). The highest lipid content was observed in the *Euglena* sp. (24.6–31%) that is comparable to the earlier reports [49] and higher values are reported in some studies [44]. The details of which are illustrated in Table 3. However, the lipid content of *Phormidium* sp. was 50% of what was reported earlier [42], and were comparable to the other studies [43] as given in Table 3. The total lipid content in *Spirogyra* sp. was comparable with the values reported in earlier studies [44] and was relatively higher than many other studies [45–48].

3.4. Comparison of lipid extraction methods

The best yield was obtained using sonication among different extraction and cell disruption techniques. Palmitic acid, stearic acid and oleic acid were commonly dominant in all the three tested algal samples. The highest amounts of these crucial fatty acids from a biodiesel perspective were obtained using sonication (Fig. 4) which is in agreement with earlier studies [25,50,51]. A

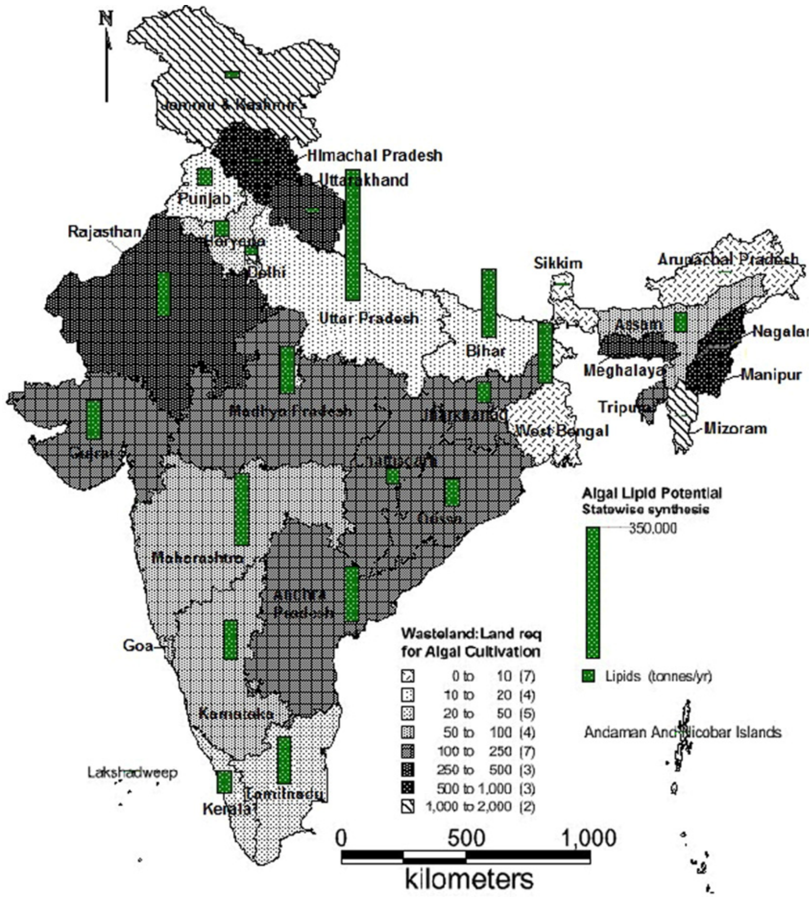


Fig. 3. Biofuel potential (t/year) of wastewater with available wasteland (for growing algae). The bar plots indicated the biofuel potential of the states and the shades (legends ascribed in the region show the ration between the wasteland to land required for algal Cultivation.

Table 3
Fatty acid composition of select algal species.

Algae	Total lipids (%)	Major fatty acids composition	Source
Euglena			
E. gracilis **(LP)**	–	[18:3(55.1%) > 16:1(9.1%) > 18:2(9%) > 16:0(8%)]	[57]
E. gracilis **(DP)**	–	[14:0(37.9%) > 16:0(29.3%) > 16:2(15.8%) > 18:2(3.5%)]	[57]
E. gracilis	27.20	[18:3(31%) > 16:0(25.2%) > 16:4(14.8%) > 18:0 + 16:3(7%)]	[49]
E. gracilis	14–20	–	[60]
Euglena **sp.**	24.6–31	[16:0(24.05%) > 14:0(20.25) > 18:0(15.36) > 18:1(6.58%)]	Present study (2011)
Spirogyra			
Spirogyra **sp.**	11.9–16.1	[18:1 > 16:1 > 16:0 > 18:2 > 18:3]	[45]
Spirogyra **sp.**	11–21	–	[44]
S. crassa	5.60	[16:0 > 18:2 > 18:3] and 20:4, 20:5(minor)	[46]
Spirogyra **sp.**	7.30	–	[47]
Spirogyra **sp.**	14.82	[18:1(33.3%) > 16:0(25.25) > 18:2(10.8%) > 14:0(6.4%)]	[48]
Spirogyra **sp.**	18.4–20	[16:0(52%) > 18:0(17%) > 18:1(16%) > 14:0(2%)]	Present study (2011)
Phormidium			
Phormidium corium	5.60	[16:0(35%) > 18:1(30%) > 16:2(14%) > 16:1 (13%)]	[58]
P. jenkelianum	4.50	[18:1(36%) > 16:0(29%) > 16:1(19%) > 18:2(11%)]	[58]
P. tenue	–	[18:0 > 18:3]	[59]
Phormidium **sp. (LP)**	11	[16:0(21%) > 18:0(12.7%) > 18:1(11.3%) > 16:1(11%)]	[43]
Phormidium **sp. (DP)**	9.40	[16:0(29%) > 18:0(12.9%) > 18:1(6%) > 16:1(4%)]	[43]
P. laminosum	–	[16:1$^{\Delta 9}$(32%) > 16:0(31%) > 18:1$^{\Delta 9}$(12%) > 16:3(9%)]	[63]
P. tenue	10.95	–	[64]
P. purpurescens	26	[16:0(39%) > 18:2 $^{\Delta 9,12}$(18%) > 18:1$^{\Delta 9}$(9%) > 18:0(5%)]	[42]
P. ambiguum	10	[16:0(9%) > 24:0(7%) > 12:0 (5%)]	[42]
Phormidium **sp.**	6.18–11.54	[16:0(40.2%) > 18:0(22%) > 18:1(21.7%) > 18:1$^{\Delta 9}$(8.8%)]	Present study (2011)

*LP-Light phase; DP-Dark phase.

number of studies on the comparative analysis of the various extraction methods have been conducted (Table 4). Algal cell disruption by maceration is one among the common and cost-effective methods for deriving lipids and has been proved to the best among other techniques [52,53]. In the present study maceration technique proved to provide better lipid yields compared to bead-beating.

Many of the other studies acquired high yields with bead milling technique. Among the various techniques applied for cell disruption as bead milling, high press homogeniser, microfluidiser homogeniser and methods as chemical permeabilisation and enzymatic disruption, the bead milling method was the most efficient [23]. However wet-milling was efficient among bead-beater, French press and sonication [22]. Other advanced cell disruption techniques were also employed in many studies viz. microwave assisted digestion [25] at supercritical conditions [54]; pulsed electric field [55]. A detailed comparative account of various cell disruption approaches and efficient methods of extraction is illustrated in Table 4.

3.5. Fatty acid composition

GC–MS analyses of the fatty acids through chromatograms illustrate peaks of the fatty-acid methyl esters in Figs. 5–7 for *Euglena* sp., *Spirogyra* sp. and *Phormidium* sp., respectively. Tables 3 and 4 lists the order and composition (saturates and unsaturates) of fatty acids.

In *Euglena* sp., the yield of important fatty acids (from a biofuel perspective) was lower than 50% but in case of *Spirogyra* sp. and *Phormidium* sp. the yield approached 90% using sonication. The important fatty acids obtained from each algae using bead beating, maceration and sonication were compared and are depicted in Fig. 3. The results are in agreement with earlier studies where palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) were recognized as the most common fatty acids contained in biodiesel providing a reasonable balance of fuel properties [56]. Earlier studies showed the accumulation of unsaturates (18:3 > 18:2) followed by saturates (16:0) as the major fatty acid component [48,57] during the light

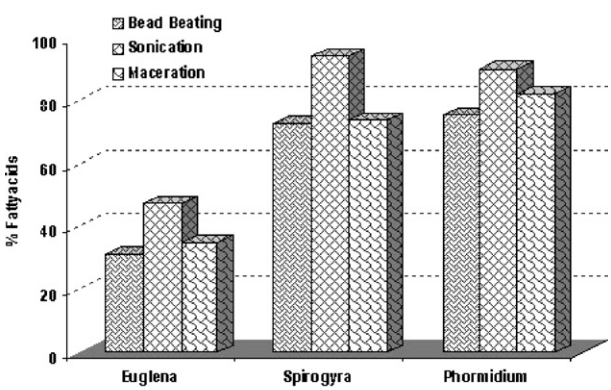


Fig. 4. Fatty acids (%) from select algae species—comparative analysis of disruption techniques.

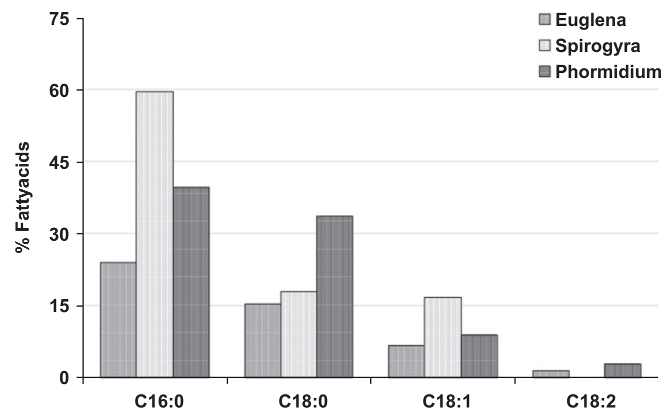


Fig. 5. Fatty acids (%) through sonication.

Table 4
Comparison of different cell disruption techniques and the efficient method for better lipid recovery.

Algae	Approaches	Efficient method	Source
Egregia menziesii, Chondracanthus canaliculatus, Ulva lobata	Mechanical homogenisation	Maceration	[52]
E. coli[a], *B. subtilis*[a], *S. cerevisiae* [b]	***Mech.***: Bead mill, sonication, high press. Homogeniser, microfluidizer homogenizer. ***Non Mech.***: Physical disruption, Chemical permeabilization, enzymatic disruption	Bead milling	[23]
Enteromorpha intestinalis, Enteromorpha clathrata, Ulva lactuca, Codium tomentosum, Hypnea valentiae, Padina gymnospora	Mechanical methods	Maceration	[53]
Spirulina maxima, Chlorella vulgaris, Scenedesmus obliquus, Dunaliella tertiolecta, Nannochloropsis, Neochloris oleabundans	Soxhlet, sonication	Sonication	[50]
Scenedesmus dimorphus, *Chlorella protothecoides*	Bead-beater, French press, sonication, wet milling	Wet milling	[22]
Scenedesmus sp.	Soxhlet, Vortex, soxhelt (*n*-hexane), sonicator (CHCl3–MeOH)	Sonication	
Botryococcus sp., *Chlorella vulgaris*, *Scenedesmus*	Autoclave, bead beating, microwave, sonication, osmotic shock	Microwave	[24]
Chlorella sp., *Nostoc* sp., *Tolypothrix* sp.	Autoclave, Sonication, osmotic shock, microwave, bead beating	Sonication	[25]
Nannochloroppsis sp.	Microwave-assisted extraction at various cond's	Supercritical cond's	[54]
Synechocystis PCC 6803	Electroporation	Pulsed electric-field	[55]
Euglena sp., *Spirogyra* sp., *Phormidium* sp.	Bead beating, maceration, sonication	Sonication	Present study, 2011

[a] Bacteria.
[b] Yeast.

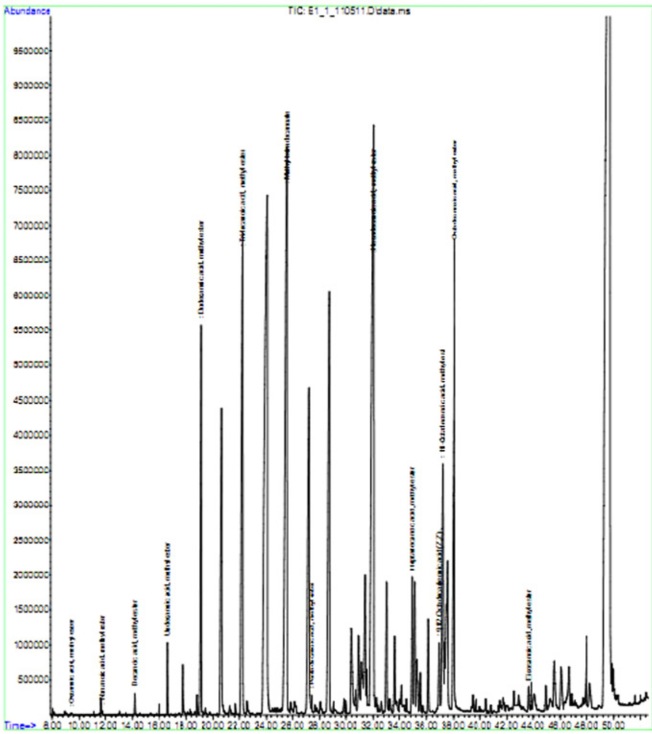


Fig. 6. FAME chromatogram—*Euglena* sp.

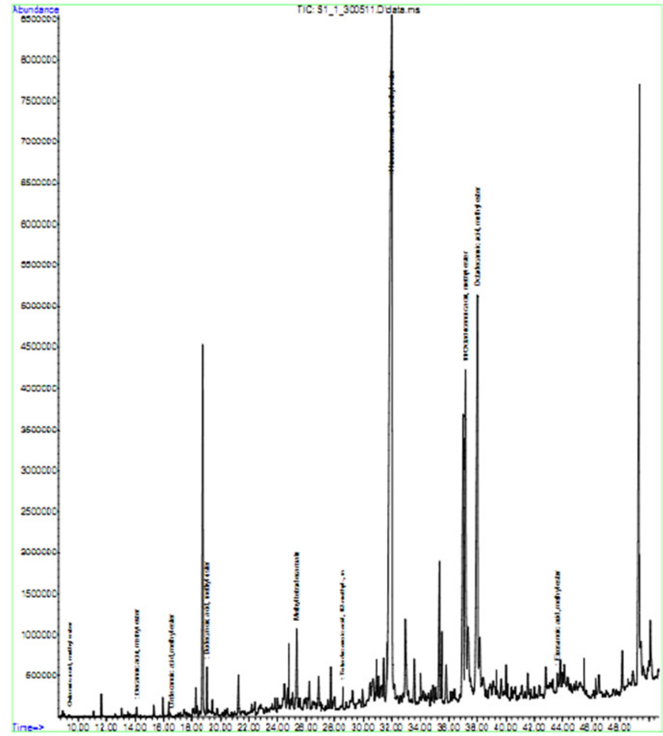


Fig. 7. FAME chromatogram—*Spirogyra* sp.

Table 5
Fatty acid compositions of algal species with different cell disruption techniques.

		Euglena			Spirogyra			Phormidium		
CN: U (%)	**Fatty acid methyl esters**	**Bead.**	**Soni.**	**Mace.**	**Bead.**	**Soni.**	**Mace.**	**Bead.**	**Soni.**	**Mace.**
C8:0	Octanoic acid, methyl ester	–	0.002	–	0.059	0.043	1.005	–	0.061	–
C9:0	Nonanoic acid, methyl ester	–	0.022	–	–	–	–	–	–	–
C10:0	Decanoic acid, methyl ester	0.128	0.23	0.081	0.235	0.183	2.706	–	0.345	–
C11:0	Undecanoic acid, methyl ester	0.565	0.688	0.407	–	0.021	–	–	–	–
C12:0	Dodecanoic acid, methyl ester	6.05	6.146	5.351	1.324	1.221	13.306	8.297	1.95	2.778
C13:0	Tridecanoic acid, methy ester	14.547	11.963	13.153	–	–	–	0.968	0.308	0.48
C14:0	Methyl tetradecanoate	26.435	20.254	23.78	2.17	2.501	9.043	11.443	2.803	5.511
C14:0	Tetradecanoic acid, 12-methyl	–	–	–	0.891	–	0.891	–	–	–
C15:0	Pentadecanoic acid, methyl ester	14.244	10.683	13.169	0.536	–	–	–	–	–
C16:0	Hexadecanoic acid, methyl ester	24.063	24.049	26.605	44.958	59.707	51.907	53.893	39.754	26.968
C16:0	7-Hexadecanoic acid, methyl esters	–	–	–	–	–	–	–	–	1.539
C16:0	9-Hexadecanoic acid, methyl esters	–	–	–	1.205	–	–	–	0.199	0.645
C17:0	Heptadecanoic acid, methyl ester	2.664	2.103	2.482	–	–	–	–	–	0.767
C18:0	Octadecanoic acid, methyl ester	4.448	15.368	6.271	12.099	17.986	22.032	21.592	33.763	10.638
C18:1	9-Octadecenoic acid (z)-methyl ester	2.389	–	4.901	21.596	–	–	–	4.94	38.49
C18:1	11-Octadecenoic acid, methyl ester (z,z)	1.041	6.584	–	–	16.753	–	–	8.888	–
C18:2	9,12-octadecadienoic acid (z,z)	1.86	1.311	2.088	–	15.805	–	2.809	6.053	–
C20:0	Eicosanoic acid, methyl ester	0.167	0.423	0.162	–	0.694	–	–	1.362	–
C22:0	Docosanoic acid, methyl esters	–	–	–	–	–	–	–	2.626	–

phase. However the experiments conducted in the dark phase yielded a very high proportion of saturates (14:0 > 16:0) compared to unsaturates (16:2 > 18:2) [46] the details of which are provided in Table 3. Other studies have showed higher oleic acid content followed by palmitate and lineolate in *Spirogyra* sp. [44,48].

Phormidium sp. comprised higher amount of palmitate, decent amount of stearate and oleate followed by a small amount of linolenic acid (Table 5). This is in agreement with the earlier studies on *Phormidium* sp. in both light and dark phases [43], where the main components of the fatty acids were saturates followed by unsaturates. In most of the studies in *Phormidium* as in the present study, palmitic acid have been consistently the major fatty acid [58,42,43] with few exceptions [59] where the dominant fatty acid are stearate (18:0) in *Phormidium tenue* and linolieate (18:1) followed by palmitate (16:0) in case of *Phormidium jenkelianum* [58]. *Spirogyra* sp. and *Phormidium* sp. with higher amount of biofuel type fatty acids has proved to be a reasonably good candidate for biodiesel production [47].

Table 6
Fatty acids with the degree of unsaturation and order of series.

Algae	Degree of unsaturation	Order of series
***Euglena* sp.**	Saturated > monoenes > dienes	C16:0 > C18:0 > C18:1 > C18:2
***Spirogyra* sp.**	Saturated > monoenes > dienes	C16:0 > C18:1(9) > C18:0 > C18:1(11) > C18:2
***Phormidium* sp.**	Saturated > monoenes > dienes	C16:0 > C18:0 > C18:1 > C18:2

Table 7
Saturated and unsaturated neutral lipids in selected algae.

Type of fatty acid	Composition
Saturates	C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0
Unsaturates	C16:1(7), C16:1(9), C18:1(11), C18:1(9), C18:2(9,12)

Palmitate was highest in *Spirogyra* sp. i.e., approximately 60% compared to *Phormidium* sp. (40%) and *Euglena* sp. (24%). Similarly, *Spirogyra* sp. had the highest oleic acid, followed by *Phormidium sp*. and *Euglena* sp. (Table 5). The results of important fatty acid composition in *Spirogyra* sp. are in agreement with earlier study where there is higher palmitate content followed by stearate [46]. The present analysis showed a total of 21 types of fatty acids and the number of fatty acids detected in *Euglena* sp. (Fig. 5), *Spirogyra* sp. (Fig. 6) and *Phormidium* sp. (Fig. 7) were 16, 13 and 15, respectively (Table 5). It was observed that in majority tetradecanoic acid (C14:0), hexadecanoic acid (C16:0), octadecanoic acid (C18:0) and eicosanoic acid (C20:0) were common among saturates and 11-octadecenoicacid (C18:1), 9,12-octadecadienoic acid (C18:2) were present in all the three populations, where as Docosanoic acid (C22:0) was present only in *Phormidium* sp. and Pentadecanoic acid (C15:0) and Nonanoic acid (C9:0) were only present in *Euglena* sp. members. Of the 23 fatty acids, nine were present in all three populations. The percentage of saturates were higher followed by mono-enes, di-enes and in all the three algal species (Table 6).

It is evident from the results that algae grown in wastewater are suitable for biodiesel production, due to their higher essential fatty acids content and biomass productivities. The present study provides an important relation of algal growth and the lipid characteristics in urban wastewaters. In this context these algae grows enormously in wastewaters and at a very high rate during the summer. They can be stated as the nature's culture to regulate wastewater nutrients at the same time providing variety of options for biomass utilisation. It is thus possible to enhance the nature's culture by careful optimisation of the required growth conditions for an efficient lipid recovery and sustainable energy generation from the algal biomass. Fatty acid analysis of the three species indicated the existence of the saturates as 16:0, 18:0 and unsaturates as 18:1 and 18:2 which are efficient biodiesel components (Table 7), indicating potential utilities of the wastewater algae *Euglena* sp., *Spirogyra* sp. and *Phormidium* sp. for better energy security together with wastewater treatment and thus foster human welfare Fig. 8.

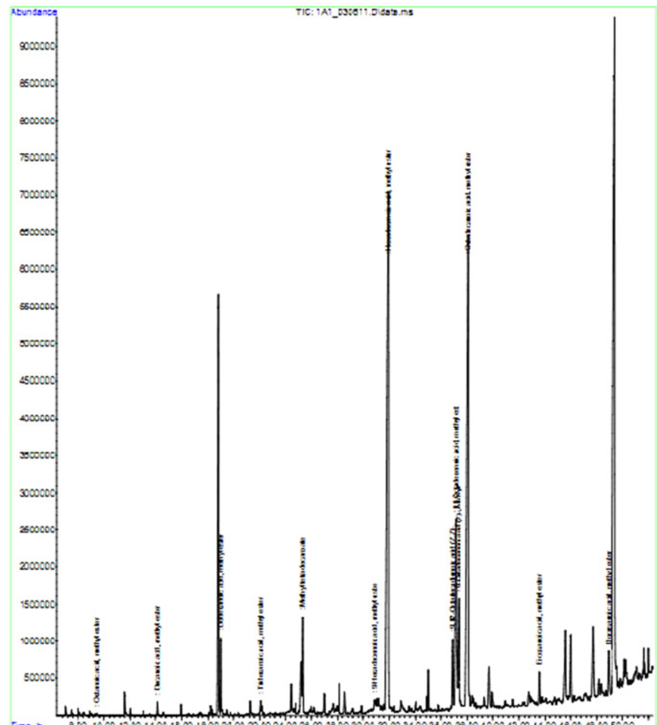


Fig. 8. FAME chromatogram—*Phormidium* sp..

4. Conclusion

The integration of municipal wastewater treatment with algal biofuel generation is an economically viable and attractive option for reducing nutrient load (C,N,P), GHG emissions, and meeting the decentralised energy demand. The efficiency of lipid extraction from algae was found to differ according to the species, cell disruption and associated extraction methods. The highest lipid was extracted from *Euglena* sp. (24.6–31%) followed by *Spirogyra* sp. (18.4–20%) and *Phormidium* sp. (6.18–11.54%). Fatty acid composition analysis showed highest C16:0 and C18:1 in *Spirogyra* sp. followed by *Euglena* sp., and *Phormidium* sp. showed highest C18:0, when the cells were disrupted through sonication. Thus of all the methods adopted for cell disruption sonication method showed the highest efficiency. Lipid productivities of *Euglena* sp. were 40 times higher than *Spirogyra* sp. and *Phormidium* sp. Higher biomass productivities of wastewater-grown algae such as *Euglena* sp. (6.52 t/ha/year) suggests algae based treatment option for removal of nutrients from wastewater as well as biofuel production for fostering the sustainable production of renewable energy.

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